Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

Issued October 22, 1909.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN 119.

A. D. MELVIN, CHIEF OF BUREAU.

STUDIES ON BLOOD AND BLOOD PARASITES.

I. OBSERVATIONS ON MAMMALIAN BLOOD WITH DARK-FIELD ILLUMINATION.

II. THE PRIORITY OF CRYPTOBIA LEIDY, 1846, OVER TRYPANOPLASMA LAVERAN AND MESNIL, 1901.

III. TRYPANOSOMA AMERICANUM N. SP., A TRYPANOSOME WHICH APPEARS IN CULTURES MADE FROM THE BLOOD OF AMERICAN CATTLE. (Preliminary Notice.)

BY

HOWARD CRAWLEY,

Expert in Protozoology, Zoological Division.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1909.

THE BUREAU OF ANIMAL INDUSTRY.

Chief: A. D. Melvin.

Assistant Chief: A. M. Farrington.

Chief Clerk: Charles C. Carroll.

Biochemic Division: M. Dorset, chief.

Dairy Division: B. H. Rawl, chief.

Inspection Division: Rice P. Steddom, chief; Morris Wooden, R. A. Ramsay, and Albert E. Behnke, associate chiefs.

Pathological Division: John R. Mohler, chief.

Quarantine Division: Richard W. Hickman, chief.

Zoological Division: B. H. Ransom, chief.

Experiment Station: E. C. Schroeder, superintendent.

Animal Husbandman: George M. Rommel.

Editor: James M. Pickens.

ZOOLOGICAL DIVISION.

Chief: B. H. Ransom.

Scientific Assistants: Harry W. Graybill, Albert Hassall, Maurice C. Hall, and Howard Crawley.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., August 26, 1909.

Sir: I have the honor to transmit herewith, and to recommend for publication as a bulletin of this Bureau, three papers concerning studies on blood and blood parasites, by Mr. Howard Crawley, expert in protozoology in the Zoological Division of this Bureau.

Respectfully,

A. M. Farrington,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.

I. Observations on mammalian blood with dark-field illumination............ 5

II. The priority of *Cryptobia* Leidy, 1846, over *Trypanoplasma* Laveran and Mesnil, 1901.. 16

III. *Trypanosoma americanum* n. sp., a trypanosome which appears in cultures made from the blood of American cattle. (Preliminary notice.)........ 21

ILLUSTRATIONS.

Page.

Fig. 1. Beaded threads in blood.. 10

2. *Cryptobia helicis* Leidy.. 18

3. *Trypanosoma americanum* n. sp.. 28

STUDIES ON BLOOD AND BLOOD PARASITES.

I. OBSERVATIONS ON MAMMALIAN BLOOD WITH DARK-FIELD ILLUMINATION.

INTRODUCTION.

In the course of a study made on mammalian blood, having for its object the cultivation in vitro of *Piroplasma bigeminum*, numbers of curious objects were seen, which, at first glance, might readily have passed for stages in the life cycle of a parasitic protozoan. It was therefore considered advisable to devote some attention to the phenomena displayed by normal blood, in order to clear up such possible sources of confusion. This work was done with dark-field illumination, and while the observations are perhaps in no case strictly original, they have been deemed worthy of presentation, if for no other reason than that they demonstrate the very high value of this method in microscopical research.

Objects seen but dimly or not at all with ordinary light are by this means brought out with perfect clearness. This is pointed out in a recent paper by Alfred C. Coles (1909). That author examined a given region of a preparation containing *Treponema pallidum* alternately with the dark-field illumination and ordinary light. This was done by changing the condensers without moving the slide. Over the dark field the organisms were plainly seen, but by transmitted light, in spite of the most careful focusing and adjusting of illumination, they remained wholly invisible.

My experience in the examination of fresh blood is quite similar, and warrants the conclusion that the use of the dark field is a sine qua non in such studies. Thus, we find reference in hematological literature to the presence of flagellated cells in fresh blood. Such, however, appear in most cases to have been looked upon as unusual occurrences and very difficult to demonstrate. This is no doubt the case when blood is studied by transmitted light, but when dark-field illumination is used flagellated cells are frequently and easily seen in fresh blood, as will presently be shown.

The apparatus used in these studies consisted of a substage condenser, arc lamp, and rheostat, the latter serving to cut down the laboratory current to 4 amperes. In the use of this apparatus the top part of the condenser, the slide, and the cover-glass must all be scrupulously clean, and the film of oil between the condenser and the slide must be free of air globules. The mount, for the sake of the observer's eyes, should also be free from air globules, since they shine with the brilliancy of the sun when the light strikes them. It is stated in the literature published by the manufacturers of the dark-field illuminating apparatus that the slides used should not exceed 1 millimeter in thickness, and this seems to be a rather important point. Frequently the condenser has to be raised or lowered a little before the conditions become what they should, the criterion being the shade of the background, which should be black.

The work was done with a $\frac{1}{12}$-inch achromatic immersion lens, stopped down with a hard rubber funnel. For the most part a No. 12 compensating eyepiece was used, giving a theoretical magnification of 1,440 diameters. Equally good pictures, however, were to be had with a No. 18 eyepiece, giving 2,160 diameters. In fact, the impression is that even much higher powers could be successfully used, since the details shown by the highest eyepieces were quite as clear as those seen with the lowest.

The blood studied was that of the cow, sheep, rabbit, guinea pig, white rat, and man. That of the cow and the sheep was drawn from the jugular vein, defibrinated, and preserved in sterile culture tubes. The media used were common beef bouillon, citrated salt solution, or salt solution alone. In a few cases no culture media were used at all.

In spite of the somewhat varied technique, the phenomena presented were throughout much the same. Citrated salt solution, however, made by mixing equal volumes of a one-half per cent solution of each of the constituents, appears to have a destructive influence on the blood cells when permitted to act upon them for any length of time. In bouillon, on the other hand, they maintained their integrity for many days.

It may also be noted that the dark-field illumination has an injurious action on living cells. This is doubtless due in part to the fact that that portion of the slide directly above the condenser is heated. The actinic rays of the electric light may also play a part. Parenthetically, it may be observed that trypanosomes perish very quickly when placed in such an environment.

PHENOMENA OBSERVED.

The phenomena observed are best treated under the following captions: (1) Blood dust; (2) beaded threads; (3) flagellated erythrocytes and free flagella; (4) bodies showing pseudopodia; (5) erythrocytes; (6) leucocytes; (7) blood plates.

BLOOD DUST.

Blood dust, so called, consists of rounded, elongated, dumb-bell or irregularly shaped particles varying in dimensions from elements 1 or 2 μ in diameter down to those just within the limits of the highest powers of the microscope. It has been described a number of times, and a résumé of the literature is given by Nicholls (1906). To this author also belongs the merit of giving us an excellent account of this element of the blood. His conclusions are that blood dust is not an element sui generis, but is of diverse origin. He gives as possible sources of origin the granules of dead or dying leucocytes, fragments of both erythrocytes and leucocytes, and finally precipitates from the plasma.

My own observations show that the dust is a constant constituent of the blood of all the animals studied. A drop of blood, either pure or in any of the several media used, shows a greater or less number of small dancing particles. These, as stated by Nicholls, are in some cases as large as 2 μ in dimensions, but they run down to particles so small that even under 2,160 diameters, and intensely lighted, they can be seen but as points of light in a ceaseless dance in the medium. There does not seem to be any means whereby the precise dimensions of such particles can be determined, but they are certainly far below the conventional and incorrect one-half micron which is often said to be the limit of microscopical resolution, for blood dust can be seen, against the dark field, with the 16 mm. objective and No. 2 compensating eyepiece, which gives a magnification of only 31 diameters. Under such powers it is easy to see that the field is full of dancing granules, although nothing can be made out as to their shape. Presumably, with this low magnification, only the larger granules are visible; that is, those approaching a size of 1 to 2 μ.

Under a magnification of 2,160, or some seventy-fold greater, particles of blood dust can be seen which are so small that they appear as mere specks of light. I am not certain that it is justifiable to regard these as only one-seventieth as large as those revealed by a magnification of 31, but they are certainly extremely minute, and probably but a small fraction of a micromillimeter in dimension.

As already stated, the larger particles of blood dust are round, oval, or rod or dumb-bell shaped. The smaller have always the appearance of solid bodies; the larger are either solid or vesicular.

While the dust was always present, it varied enormously in quantity in different animals and in the same animal at different times and under different conditions. Nicholls believes it to be more abundant after a meal. It was also observed that, in general, the longer a drop of blood was kept on a slide the greater became the quantity of dust. This goes to show that it increases in quantity as the blood degenerates, which necessarily takes place if it be kept on a slide as a wet mount. On the other hand, a bouillon culture of cow's blood, kept for forty-seven days at room temperature, showed the erythrocytes much as they had looked on the first day, and approximately the same quantity of dust.

Since, as stated above, the object in making these researches had to do with the clearing up of possible sources of confusion with regard to the presence of protozoa, the question of the origin of the various elements seen was only of secondary interest and was not pushed to any conclusive end. Nevertheless certain observations were made which indicated at least one of the sources of blood dust. It was frequently seen that the particles tended to collect around the erythrocytes. In one case the erythrocytes—the blood being that of the guinea pig—were seen to be studded around their peripheries with a mantle of small granules, evidently of the same nature as those which were simultaneously dancing in the plasma. But in the guinea pig, cow, and man it was at times possible to make sure that there were free, motile granules *within* the red cells. The proof that they were within rested upon two points. In the first place, by shifting the focus from the upper to the lower surface of a given cell there was consecutively a less, a greater, and a less number of granules brought into view. That is, when the focal plane was on an optical section of the erythrocyte, the greatest number of granules was seen, which seems to shut out the possibility that they were on the surface. Secondly, when attention was concentrated upon a given red cell, it could be seen that the granules contained within its border showed a far less lively dancing movement than those which were free in the plasma. The only explanation seems to be that their movements were hampered by the bounding wall of the red cell. It therefore seems permissible to conclude that a certain portion of blood dust consists of broken-up erythrocytes or fragments derived from them.

After an experience of a couple of months with the dark-field illumination I became greatly impressed with its capabilities, and preparations were made of blood serum and bouillon which had been passed through a Chamberland B filter. Such filtrates showed the presence of great numbers of dancing particles, of the apparent size of the smaller particles of blood dust. It thus became evident that organic liquids, commonly looked upon as mere solutions and absolutely sterile to all bacteriological tests, contained corpuscular elements large enough to be resolved by the dark-field illumination. Nicholls also observes that serum taken from serous cavities contains the dust. We therefore see that a certain portion of the blood dust is not "blood" dust at all, in the sense of its being peculiar to blood, but it is rather to be regarded in the nature of a precipitate and peculiar to all organic fluids. This fact has a certain bearing on the problem of the so-called "ultramicroscopic" germs of certain diseases; that is, the discovery of corpuscular elements in virus would be no warrant for concluding them to be the cause of the disease in question. It would be necessary to differentiate them from the so-called "dust," an undertaking which strikes one as rather discouraging.

BEADED THREADS.

These consist of strings of beads, or of threads showing septations, the appearance being somewhat that of a string of streptococci. While they may be seen by transmitted daylight, it is only in preparations studied over the dark field that their abundance becomes evident. They occur either attached (fig. 1, *a*) to erythrocytes or free in the liquid.

In the former case there may be one or many attached to the same cell, and they vary a good deal in appearance and dimensions. At times they are extremely delicate, with the septations scarcely to be made out, and here they closely resemble the "flagella" to be described below. This condition by gradual stages leads up to one wherein we find attached to a red cell a very evident string of beads. In nearly all cases the terminal bead is somewhat larger than the others. In length these attached strings may be very short, consisting of only two or three beads, or they may be two or three times longer than the diameter of an erythrocyte. The width of such strings is not easily measured, but it probably never much exceeds 1μ, and may be much less.

In all cases these attached strings are motile. The movement may be spoken of as flagellar, but it differs from a typical flagellar movement in that it is more regular and its rapidity more uniform. It more nearly resembles that of the tentacles of a sea anemone or of the various members of a colony of fresh-water polyzoa. The cause is doubtless the slight trembling of the erythrocytes—always to be noted in fresh blood mounts—combined with whatever influence it may be that produces Brownian movement. It is sometimes more, sometimes less, lively, but never either at all slow or especially quick.

In addition to the beaded threads attached to the red cells, abundant specimens are to be seen free in the liquid. The very exact resemblance between the free and the attached forms points to a common origin. The former, however, reach a much greater length, and in nearly all cases the two terminal beads are larger than the others. The length of one of these threads in human blood was 25 μ, and there were others seen which considerably exceeded this measurement.

In their movements the free specimens are like those attached, in so far as the movements of a free body may be correlated with those of one which is fixed. There is a constant wavering and trembling of the whole thread and in addition a bending and looping movement. Fairly intricate figures are often formed by those which are being carried about by currents in the liquid.

There are a few observations which point to the mode of origin of these beaded threads. Frequently in the blood of most of the animals studied there could be seen groups of three or more vesicular, rounded bodies, varying in diameter from nearly that of a red cell downward. They had all the appearance of undoubted red cells, and my interpretation is that they were red cells in process of fragmentation. As a rule such groups showed one vesicle much larger than the others. There were also seen red cells to which were attached longer or shorter chains of little vesicular bodies, round or oval in contour. In general, the vesicles composing such chains were much of the same size in the case of the same cell, but showed a considerable variation for different cells. But this is not always the case, as shown by figure 1, *b* and *c*, drawn from appearances seen in the blood of the cow.

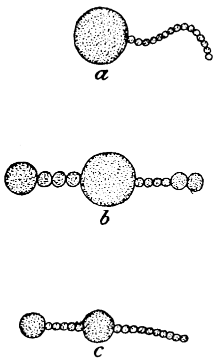

FIG. 1.—Beaded threads in blood. *a*, erythrocyte with attached beaded thread; *b*, erythrocyte to which are attached two strings of vesicles; *c*, element consisting of vesicles and beaded threads. Magnification about 1,100.

In the former of these we have an erythrocyte to which is attached two strings composed of the vesicles just described. Of these, some are relatively large, others are nearly as small as the beads. In figure 1, *c*, is shown a system composed of two large vesicles and two strings of beads. The latter, which always appeared to be solid, might be interpreted as derived from the former by a process of budding or by a mere contraction in size. It is therefore not unreasonable to suppose that the beads are derived from the vesicles, while the latter are budded-off portions of the stroma of the red cell.

The threads were first discovered in a bouillon culture of cow's blood six days old. They were also found in a like culture forty-seven days old. They appeared, however, in preparations of blood of all the animals used. But whereas blood dust is constantly present in blood as soon as it is drawn, it was not possible to get any evidence that this is so for the threads. The quickest observed to appear was in human blood, when one was seen seven minutes after the blood was drawn. It was also noticeable that in blood drawn on slides, either clear or in citrate, free threads were seen much sooner than those attached, and that in general it was easier to find them the longer the mount was kept. But the number of free threads and of corpuscles to which they were attached is very small in proportion to the whole number of red cells present, and hence the finding of free threads sooner than those attached might be due merely to accident.

FLAGELLA.

In bovine blood known to harbor *Piroplasma bigeminum*, kept in bouillon in the incubator for six days, erythrocytes were found to which were attached very delicate flagella, in constant, rather lively, lashing movement. This suggested the possibility that these represented the microgametes of *P. bigeminum*, and that in *Piroplasma* reproductive processes occur similar to those which take place in *Halteridium*. Unfortunately for this interesting hypothesis, exactly the same sort of flagellated cells were observed in blood from a northern cow which had, so far as was known, never been exposed to infection with *Piroplasma*, and later in that of all the other animals used.

But they were best demonstrated in sheep's blood, drawn direct from the jugular vein into sterile test tubes containing citrated sodium chlorid solution. In mounts from this material the appearances were at times astonishing, the entire field being filled with delicate, motile threads, either free or attached to the red cells. In the latter case the flagella usually arose from little V-shaped projections, and a given cell might support several. Their thickness was infinitesimal, and the field had to be very black and the light intense before they could be easily seen. In length they exceeded by six to eight times the diameter of the blood corpuscles.

In blood from the other animals flagellated red cells and less frequently free flagella could usually be found, but they were never present in anything like such abundance. The assumption is natural that the free flagella were merely those which had broken loose from their attachment to the cells, although this was never actually observed to take place.

I was not able to satisfy myself that the flagella were the end stage of the beaded threads. There is not much doubt, however, that they represent some sort of destructive process. As stated, they were most abundant in blood preserved in citrated sodium chlorid solution, which, for other reasons, was shown to have a destructive influence. They have been seen before, and are figured by Lignières (1900a).

BODIES SHOWING PSEUDOPODIA.

Koch (1906) and Kleine (1906) describe and figure bodies with long pseudopodia which they interpret to be stages in the evolution of *Piroplasma*. Koch illustrates these in his Plate I, figures 7–16, the material having been obtained from engorged ticks taken from cattle suffering with Texas fever. They were seen only during the first day after removal from the cow. A slow ameboid creeping movement is described, with an alternate increase and decrease in the length of the pseudopodia. On Plate 3, figures 51–63, Koch figures similar bodies as developmental stages of the parasite of East Coast fever, *P. parvum.*

Kleine took defibrinated blood from dogs infected with *Piroplasma canis,* and cultured it at 27° C. after dilution with one-half per cent sodium chlorid solution. Preparations taken from such cultures eighteen to forty-eight hours after making showed bodies in all respects like those described and figured by Koch. (See Kleine, Pl. 4, figs. 6–13; Pl. 5, figs. 14–21.) In fresh mounts they were seen to change shape and the pseudopodia to increase and decrease in length and numbers. Christophers (1907), in his elaborate article on the cycle of *P. canis* in the tick, was not able to find bodies in any way resembling these.

In cultures, in either bouillon or citrated salt solution, of blood from either northern or southern cattle there were found bodies somewhat smaller than erythrocytes, round or oval, which bore from one to several long stiff processes. These bodies were not observed to move spontaneously nor to undergo any conspicuous changes of shape. A false impression of life was given them, however, by the fact that the processes bent and wavered and underwent changes in length. The bodies themselves were either homogeneous or granular.

They were first found in blood from a southern cow known to harbor *Piroplasma bigeminum,* and were identified as being the same as the bodies described by Koch and Kleine. Their subsequent discovery in blood from northern cattle showed, however, that they could have nothing to do with the Texas fever parasite, nor were they found in preparations made from engorged ticks.

It may therefore be concluded that they are only greatly altered blood cells; erythrocytes more probably than leucocytes, for the "pseudopodia," although very long and often very delicate, have something of the appearance of very much elongated thorns and do not resemble those often seen emerging from undoubted leucocytes. It may finally be pointed out that the German investigators fail to state whether their results were checked with material known not to contain *Piroplasma.*

ERYTHROCYTES.

As already stated, in bouillon cultures of cow's blood the red cells maintain their integrity for at least forty-seven days, but a drop of blood, either pure or mixed with citrate solution and studied with the dark-field illumination, shows quite rapid alterations.

Erythrocytes when first seen under such conditions are either perfectly circular or more or less crenated. In either case, however, the periphery appears as an intensely bright band with a more or less yellowish color. In the circulation red cells are no doubt always circular, while crenation is the expression of some disturbance in the osmotic pressure between the cell and the medium in which it floats. But when pure blood either from man or from the guinea pig was kept over the dark field the originally bright red cells were observed to undergo the following changes: In those which were crenated when first brought under observation the irregular contours gradually became smoothed out, the cell finally showing as a bright circle, or rather as a bright thick ring. Then more or less suddenly they became pale, turning each to a transparent shell bounded by a faint bluish ring. These shells would sometimes fade out of sight altogether, but they mostly remained as such, and frequently there appeared within them dancing granules. Changes of this sort would take place within a few minutes, but were not always displayed by all of the cells under observation. In a drop of human blood, kept for twenty-four hours, the red cells were all reduced to these pale shells, most of which showed dancing granules within.

Curious phenomena were observed when blood was treated with one-half per cent solution of chlorate of potash. Here, as already noted, the originally bright crenated red cells gradually became perfectly circular, with a pale periphery. But in certain of these, the first instance being noted fifty minutes after the preparation was placed under the microscope, there appeared within the center a shimmer of light, and as this was watched it gradually resolved itself into a mass of excessively minute granules in a ceaseless lively dance. By slow degrees the granules became larger and larger, their movements meanwhile becoming less energetic, and finally the originally empty shell was filled with a solid mass of very bright motionless granules. This stage was reached in some cases within an hour or two, some cells undergoing the change much more rapidly than others.

In one or two cases in human blood a process somewhat the reverse of this was observed. Red cells were seen in which were a few quite large granules, or more probably little vesicles, showing a slight motility. These bodies drew together in the center of the cell and gradually fused to constitute a very irregular body, which, in its turn, became round, oval, or kidney-shaped, with smooth outlines. The outlines of these bodies were observed to undergo slight changes, and the bodies themselves to move slightly within the red cells. The appearance presented was of a cell within a cell, and although such a genesis as above described was seen in but a few cases, such elements within erythrocytes were often noted. The modus operandi suggested that we had to do with globules of either a liquid or a gas.

But several times while such cells were being watched the central body or vesicle was seen to grow smaller and then very quickly to transform itself into a little nebulous cloud and as quickly to fade into nothingness. I am not prepared to explain the meaning of this phenomenon.

LEUCOCYTES.

As is well known, leucocytes are much less hardy than erythrocytes, although they live in culture tubes for a number of days. Under the severe condition above the dark-field condenser, however, they quickly perish. In an observation made on rabbit's blood the small lymphocytes were seen rapidly to disintegrate, becoming pale shells with a few dancing granules within. The polymorphonuclear leucocytes of man run down rapidly to masses of granular débris, while the nucleus in each case breaks up into a number of round bodies with a granular content. Nevertheless they can be kept intact long enough to observe very interesting phenomena. Living leucocytes throw out pseudopodia, processes intermediate between pseudopodia and flagella, and finally actual flagella, although this last was rather rare.

The pseudopodia were of the heliozoan type, enormously long, delicate strands, which underwent rapid changes in length, swung to and fro like a pendulum, and at times lashed. The heat of the dark-field illumination evidently stimulates leucocytes to great activity, and they were observed to travel rapidly about the field, either threading or forcing their way through the masses of erythrocytes. Such cells as they progressed became somewhat elongated, with the rear end pulled out into a sort of tail, from the end of which protruded a bundle of filamentous pseudopodia. In its advance the cell would frequently make rather sharp turns to the right or left, whereupon the entire congeries of pseudopodia would be quickly swung through an extensive arc. Further, individual pseudopodia could be seen to swing through wide arcs, often to bend into curves, to crinkle and waver, and to lash like flagella. Also as the pseudopodia were pulled along behind the leucocyte they would become attached to erythrocytes and greatly stretched. Finally, the strain becoming too great, they would break loose, snap back toward the leucocyte, curling up like elastic threads, the red cells to which they had been attached simultaneously showing a recoil.

BLOOD PLATES.

Blood plates are always to be seen in freshly drawn blood, and for a time in cultures. In the former they may be as numerous as the red cells. They are not favorable objects, however, for the dark-field illumination, and are best studied by transmitted light.

In form they are often tailed, and it is possible that an observer wholly unfamiliar with the appearance of flagellates might confuse them with such. But they were not seen to display spontaneous movements, although a false appearance of this was occasionally given as they were carried about by currents in the mount. Stained preparations showed them both within and emerging from erythrocytes.

BIBLIOGRAPHY.

CHRISTOPHERS, S. R.
1907.—*Piroplasma canis* and its life cycle in the tick <Scient. Mem. Off. Med. & San. Dept. Govt. India, Calcutta, n. s. (29), 83 pp., figs. 1–4, charts 1–7, pls. 1–3.

COLES, ALFRED C.
1909.—*Spirochæta pallida:* Methods of examination and detection, especially by means of the darkground illumination <Brit. M. J., Lond. (2523), v. 1, May 8, pp. 1117–1120.

KLEINE, F. K.
1906.—Kultivierungsversuch der Hundepiroplasmen <Ztschr. f. Hyg. u. Infektionskrankh., Leipz., v. 54 (1), 26, Juli, pp. 10–16, pls. 4–5, figs. 1–28.

KOCH, ROBERT.
1906.—Beiträge zur Entwicklungsgeschichte der Piroplasmen <Ztschr. f. Hyg. u. Infektionskrankh., Leipz., v. 54 (1), 26, Juli, pp. 1–9, pls. 1–3, figs. 1–67.

LIGNIÈRES, JOSEPH.
1900 a.—La "tristeza," ou malaria bovine dans le République Argentine, vii+172 pp., 16 pls. 8°. Buenos Ayres.

NICHOLLS, ALBERT G.
1906.—On the nature and significance of the so-called "dust-bodies" of the blood. (Blutstäubchen or Hæmokonien of H. F. Muller). <Proc. & Tr. Roy. Soc. Canada [Ottawa] (1905), 2. s., v. 11, Tr., Sect. 4, pp. 3–19, figs. 1–3.

II. THE PRIORITY OF CRYPTOBIA LEIDY, 1846, OVER TRYPANOPLASMA LAVERAN AND MESNIL, 1901.

In the Proceedings of the Academy of Natural Sciences of Philadelphia for September, 1846, Dr. Joseph Leidy (1846 a) describes a new genus and species of "Entozoa" as follows:

> *Cryptobia.* Animal minute; form exceedingly proteoid; internal organization cellular or granular.
>
> *C. helicis.* Colorless; form, ordinarily elongate, ellipsolid, fusiform, or ovate; caudated, caudæ opposite, one longer than the other. Internal granular structure consisting of two large cells and numerous minute granules. Total length from the one hundred and twenty-sixth to the one hundredth of a line. Habitat, the vessie copulatrice or spermatheca of *Helix albolabris* [*Polygyra albolabris* (Say)], *Helix tridentata* [*Polygyra tridentata* (Say)] and *Helix alternata* [*Pyramidula alternata* (Say)].
>
> The name of this genus is derived from *κρυπτος*, hidden, and *βιοω*, to live. This singular entozoon in its general appearance and organization appears to be intermediate between *Cercaria seminis* and *Filaria.* Its varied form and movements are curious to observe; at one moment globular, then oval, ovate, fusiform, sigmoid, crescentic, etc., it appears as if it would outvie the kaleidoscope in its changes. The motions are vibratile, rotary, with a lateral progression, or whirling in circles like the insect *Gyrinus.*

This article is illustrated by a large number of small text figures, which show that Leidy was dealing with an organism fundamentally fusiform and which possessed a flagellum at either end. One of his figures shows a bundle of four individuals in which the flagella from one end are joined by their tips.

In the same journal for August, 1847 (Leidy, 1847 d), we find it stated that "Doctor Leidy requested permission, which was given, to change the name of a new genus of Entozoa, described by him in vol. 3, No. 5, of the Proceedings, from that of *Cryptobia* to *Cryptoicus*, the former name having been preoccupied."

In the same year (Leidy, 1847 f) a description of the animal is given in Latin, but this is nothing more than a translation of the account quoted above. We learn, however, that in addition to the three hosts already named, it was also found in *Helix elevata* [*Poly-*

gyra elevata (Say)], *Helix thyroidus* [*Polygyra thyroidus* (Say)], and *Bulimus decollatus* [*Rumina decollata* (Linné)], and that—

> This singular entozoon is a polygastric animalcule * * *. Sometimes it is collected in bunches adhering by the end of one of the cauda to each other, and frequently it may be observed to contract upon either of the long cellules, causing them to project beyond the outline of the animal * * *. In the collapsed state of the genitalia of Helices I could not detect the Cryptoicus.

Diesing (1850 a), vol. 1, p. 45, calls the parasite *Bodo* (*Cercomonas*) *helicis*, appending his own name. After giving the names of the three snails first mentioned by Leidy as hosts, and the locality as Philadelphia, he adds "*Helix nemoralis,* Vindobonæ, Augusto (Leidy)." The inference here is that Leidy found the parasite in *H. nemoralis,* at Vindobonæ, in August, but Leidy himself is silent.

Later, Leidy (1851 d and 1856 b) accepts Diesing's action in placing the animal in the genus *Bodo.*

Keferstein and Ehlers (1860) describe an "infusorian" in the bursa copulatrix of *Helix pomatia* Linné. It is present in great numbers. The organism is spindle shaped, bearing in front a lashing flagellum, while behind it is pulled out into a process about 8 μ long. The length is given as 20 μ, the width as 4 μ. The organisms are not round but flattened, and most of them show a longitudinal ridge. The content is finely granular, and after treatment with acetic acid shows an evident nucleus. The movements are mostly trembling and wavering, with a rotation on the longitudinal axis. Progression is in nearly a straight line. Occasionally some individuals were seen to creep in the manner of *Euglena.*

For close on to half a century *Cryptobia* remained undisturbed by science, but in the present year Friedrich (1909) picked it up in the "receptaculum seminis" of *Helix pomatia* L., and gives a detailed account of it. Following this, the animal possesses two flagella, of which the posterior makes up the border of an undulating membrane. Both arise from the kinetonucleus, which is of approximately the same size as the trophonucleus. Friedrich, after going into the history of the animal, decides that it is the same as that found by Leidy, and calls it *Trypanoplasma helicis* Leidy.

Clearly, however, if the later authors were dealing with the same animal as Leidy, the rules of priority compel the use of the name which he gave, and the generic name *Cryptobia* supplants that of *Trypanoplasma.* The only possible question which might arise would be whether or not *Cryptobia helicis* and *Trypanoplasma borreli* (the type species of *Trypanoplasma*) are congeneric.

In order to determine this point, living specimens of several species of snails were obtained from the neighborhood of Washington, D. C., and in *Polygyra albolabris* the flagellate was found. It is shown in figure 2. From this it will be seen that the animal possesses two flagella, which arise from two minute granules in close proximity to the kinetonucleus. One of these flagella forms the edge of an undulating membrane. The kinetonucleus is of much the same size as the trophonucleus, but it may vary a good deal in shape, being at times crescentic. Friedrich considers the structure to be a good deal the same in both kinetonucleus and trophonucleus, but says that in the former the stain is generally so intense that the details are obscured. Nuclear structure, however, depends so much upon the conditions, and it is yet so obscure in the case of the Trypanosomidæ, that it is not advisable to use it as a diagnostic character of value.

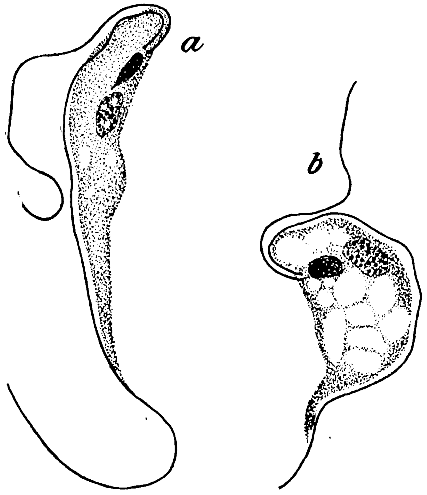

FIG. 2.—*Cryptobia helicis* Leidy. *a*, typical form, × 2,467; *b*, short, broad form, possibly somewhat degenerate, × 2,467. (Figures made from camera outlines.)

Laveran and Mesnil (1907) define the genus *Trypanoplasma* as follows:

> *Trypanoplasma*, Laveran and Mesnil, 1901 (amended 1904): Flagellates with elongated body, presenting laterally an undulating membrane, whose thickened edge is prolonged posteriorly as a flagellum, and turns back anteriorly to reach a mass (centrosome) which is as large as, and has up to a certain point the same structure as, the nucleus. An anterior free flagellum has its origin in the same mass. Multiplication is probably by binary equal longitudinal division.

This definition evidently covers the parasite of the snail. Further, a comparison of figure 2, *a* and *b*, with the drawings of *T. borreli* as given by Woodcock (1909, p. 215) shows plainly enough that that animal and *C. helicis* are congeneric. It may further be added that Friedrich reports longitudinal division, a conclusion that is confirmed by my own material. Hence the name *Trypanoplasma* must give way to that of *Cryptobia*.

This name, originally used by Leidy, was afterwards abandoned by him on the score of its preoccupancy by *Cryptobium*. But while *Cryptobium*, and *Cryptobias* as well, were used in 1830 and 1834, respectively, for beetles, this is no warrant for abandoning *Cryptobia*. For although these three names are very similar, they are not identical, and hence can not be regarded as homonyms.

The synonymy of the genus *Cryptobia*, and of the several species belonging in this genus, is given in the following table:

Genus CRYPTOBIA Leidy, 1846.

1846. *Cryptobia* Leidy, 1846a, p. 100 (type *C. helicis* Leidy 1846). (Not *Cryptobium* Mann, 1830, Coleoptera; not *Cryptobias* Serv., 1834, Coleoptera; not *Cryptobia* Desh., 1863, Mollusca.)
1847. *Cryptoicus* Leidy, 1847d, p. 239 (*Cryptobia* renamed).
1891. *Trypanomonas* [a] Labbé, 1891c, p. 231 (type *Trypanomonas danilevskyi* Labbé, 1891).
1901. *Trypanoplasma* Laveran and Mesnil, 1901i, p. 672–673 (type *T. borreli* L. & M., 1901).

Cryptobia helicis Leidy, 1846.

1846. *Cryptobia helicis* Leidy, 1846a, p. 100, figs.—Leidy, 1847f, p. 67, figs.
1847. *Cryptoicus helicis* Leidy, 1847f, p. 67, figs.
1850. *Bodo helicis* Diesing, 1850a, p. 45.—Leidy, 1851d, p. 284.—Leidy, 1856b, p. 42.—Kent, 1880c, v. 1, p. 256, pl. 14, figs. 12, 13.—Doflein, 1901a, p. 73.
1909. *Trypanoplasma helicis* Friedrich, 1909, pp. 363–395, figs. 1–48.

Cryptobia abramadis (Brumpt, 1906).

1906. *Trypanoplasma abramadis* Brumpt, 1906, p. 164.

Cryptobia barbi (Brumpt, 1906).

1906. *Trypanoplasma barbi* Brumpt, 1906, p. 164.

Cryptobia borreli (Laveran & Mesnil, 1901).

1901. *Trypanoplasma borreli* Laveran & Mesnil, 1901i, p. 672, fig. 4; p. 673.

Cryptobia cyprini (Plehn, 1903).

1903. *Trypanoplasma cyprini* Plehn, 1903a, pp. 175–181, pl. 2.

Cryptobia danilevskyi (Labbé, 1891).

1891. *Trypanomonas danilevskyi* Labbé, 1891c, pp. 229–231, figs.

Cryptobia guernei (Brumpt, 1905).

1905. *Trypanoplasma guernei* Brumpt, 1905, p. 322, fig. 34 (2); p. 323; p. 330, fig. 38 (1).

Cryptobia intestinalis (Léger, 1905).

1905. *Trypanoplasma intestinalis* Léger, 1905e, p. 512.

Cryptobia truttæ (Brumpt, 1906).

1906. *Trypanoplasma truttæ* Brumpt, 1906, p. 164.

Cryptobia varium (Léger, 1904).

1904. *Trypanoplasma varium* Léger, 1904s, p. 345.

Cryptobia ventriculi (Keysselitz, 1906).

1906. *Trypanoplasma ventriculi* Keysselitz, 1906, p. 37, figs. 46–48.

---

[a] Labbé credits this name to Danilevsky, but the latter author (1889a, p. 66) used it merely to designate young stages of trypanosomes, and hence did not establish it as a valid generic name.

BIBLIOGRAPHY.

BRUMPT, E.

1905.—Trypanosomes et trypanosomoses <Rev. scient., Par., an. 42, 5. s., v. 4 (11), 9 sept., pp. 321-332, figs. 34-41.

1906.—Mode de transmission et évolution des trypanosomes des poissons. Description de quelques espèces de trypanoplasmes des poissons d'eau douce. Trypanosome d'un crapaud africain <Compt. rend. Soc. de biol., Par., 58 an., v. 60, v. 1 (4), 2 fév., pp. 162-164.

DANILEVSKY, VASILI IAKOVIEVICH.

1889 a.—La parasitologie comparée du sang. 1. Nouvelles recherches sur les parasites du sang des oiseaux. 93 pp., 3 pls., 75 figs. 8°. Kharkoff.

DIESING, KARL MORITZ.

1850 a.—Systema helminthum. v. 1, xiii pp., 1 l., 679 pp. 8°. Vindobonæ.

DOFLEIN, FRANZ.

1901 a.—Die Protozoen als Parasiten und Krankheitserreger, nach biologischen Gesichtspunkten dargestellt. xiii + 274 pp., 220 figs. 8°. Jena.

FRIEDRICH, LUDWIG.

1909.—Ueber Bau und Naturgeschichte des *Trypanoplasma helicis* Leidy <Arch. f. Protistenk., Jena, v. 14 (3), pp. 363-395, figs. 1-48.

KEFERSTEIN, W.; & EHLERS, ERNEST.

1860.—Beiträge zur Kenntnis der Geschlechtsverhältnisse von *Helix pomatia* <Ztschr. f. wissensch. Zool., Leipz., v. 10, pp. 253-270, pl. 19.

KEYSSELITZ, G.

1906.—Generations- und Wirtswechsel von *Trypanoplasma borreli* Laveran et Mesnil <Arch. f. Protistenk., Jena, v. 7 (1), pp. 1-74, figs. 1-162.

LABBÉ, ALPHONSE.

1891 c.—Note sur un nouveau parasite du sang (*Trypanomonas danilevskyi*) <Bull. Soc. zool. de France, Par., v. 16 (8), oct., pp. 229-231, 1 fig.

LAVERAN, CHARLES LOUIS ALPHONSE; & MESNIL, FELIX.

1901 i.—Sur les flagellés à membrane ondulante des poissons (genres *Trypanosoma* Gruby et *Trypanoplasma* n. gen.) <Compt. rend. Acad. d. sc., Par., v. 133 (18), 28 oct., pp. 670-675, figs. 1-4.

1907.—Trypanosomes and trypanosomiases. Translated and much enlarged by David Nabarro. xix + 538 pp., 81 figs., 1 pl., 16 figs. 8°. London.

LÉGER, LOUIS.

1904 s.—*Trypanoplasma varium*, n. sp., parasite du sang de *Cobitis barbatula* L. [Read 5 nov.] <Compt. rend. Soc. de biol., Par., v. 56 (30), 11 nov., pp. 345-347.

1905 e.—Sur la présence d'un *Trypanoplasma* intestinal chez les poissons [Read 18 mars] <Compt. rend. Soc. de biol., Par., v. 58 (11), 24 mars, pp. 511-513.

LEIDY, JOSEPH.

1846 a.—Description of a new genus and species of Entozoa [Read Sept. 29] <Proc. Acad. Nat. Sc. Phila. (1846-47), v. 3 (5), Sept.-Oct., pp. 100-101, figs.

1847 d.—[*Cryptobia* changed to *Cryptoicus*.] [Secretary's abstract] <Proc. Acad. Nat. Sc. Phila. (1846-47), v. 3 (10), July-Aug., p. 239.

1847 f.—Miscellanea zoologica. 1. Description of a new genus and species of Entozoa. *Cryptobia helicis* <J. Acad. Nat. Sc. Phila. (1847-50), 2. s., v. 1 (1), Dec., pp. 67-68, figs.

1851 d.—Corrections and additions to former papers on helminthology, published in the Proceedings of the Academy <Proc. Acad. Nat. Sc. Phila. (1850-51), v. 5 (11), Sept.-Oct., pp. 284-290.

1856 b.—A synopsis of Entozoa and some of their ectocongeners observed by the author <Proc. Acad. Nat. Sc. Phila., v. 8 (1), Jan.-Feb., pp. 42-58.

PLEHN, MARIANNE.

1903 a.—*Trypanoplasma cyprini*, nov. sp. <Arch. f. Protistenk., Jena, v. 3 (2), pp. 175-180, pl. 12, figs. 1-18.

WOODCOCK, H. M.

1909.—The Protozoa. Section G: The hæmoflagellates, and allied forms. (*In* Lankester, Edwin Ray. A treatise on zoology. Part 1 (1), pp. 193-273, 40 figs. 8°. London.)

III. TRYPANOSOMA AMERICANUM N. SP., A TRYPANOSOME WHICH APPEARS IN CULTURES MADE FROM THE BLOOD OF AMERICAN CATTLE. (PRELIMINARY NOTICE.)

HISTORICAL.

In a postscript to his celebrated article on the blood parasites of the Little Owl, Schaudinn (1904) details some information received from Kossel and Weber in a personal communication. The last-named investigators, in Finland, in 1900, had made a smear of blood, taken from a cow dying of hemoglobinuria. In this smear, in addition to examples of a *Piroplasma,* which they identified as *Piroplasma bigeminum,* there was found a trypanosome, considerably smaller than *T. evansi.* Schaudinn examined this preparation, and after comparing the morphology of the trypanosome with that of *Piroplasma bigeminum* states that it probably constitutes a stage in the life history of *P. bigeminum* similar to that taken by the trypanosome of *Halteridium* in the life history of that blood parasite. His belief was based on the size, character of the cytoplasm, and nuclear relations of the two phases found in Kossel and Weber's preparations.

The year before, Kossel and Weber had made smears of the intestinal contents of ticks taken from sick cattle. On account of the above discovery these smears were restained and studied, and in them were found trypanosome-like stages similar to those found in the blood of the cow. Hence, with Kossel and Weber, Schaudinn accepts as a working hypothesis that the evolution of *P. bigeminum* and *P. canis* takes place in a manner similar to that of *Halteridium.*

Bowhill and Le Doux (1904), in a paper on malignant jaundice of the dog, refer to endoglobular parasites which show long, flagella-like processes. It is also stated that one of them (Bowhill) has observed flagellated forms of a *Piroplasma,* which they call *P. bovis.* They write: "The parasite consists of an enlarged elongated portion running into a delicate 'flagellum' upon which, on close examination, may be seen two minute bulbous protuberances."

Bowhill (1905), studying equine piroplasmosis, finds, free in the blood stream, parasites which "consist of a delicate, pear-shaped head, possessing a clearly defined red-stained karyosome, and a long flagellum ending in a bulbous protuberance." He adds that these flagellated forms may be within a red blood cell, or half in and half out. The flagellum may be 3.5 μ in length or even more.

A glance at the figures given by these authors shows, however, that they are not dealing with flagella at all, but with processes resembling pseudopodia. This is pointed out by Minchin (1909), who writes (p. 89):

> I suggest that the "flagellum" is probably to be interpreted as a pseudopodium, by means of which the parasite may quit a corpuscle, and by means of which it may, when free, attack and enter another corpuscle. It is not possible, however, to give a final judgment upon this point until the so-called flagellum has been seen and studied in the living condition.

Miyajima (1907) gives what seems to be excellent evidence in support of the doctrine that trypanosomes and Hæmosporidia are but phases in the life history of the same organisms. Cultures made in common bouillon of blood from Japanese cattle parasitized with a species of *Piroplasma* presently developed trypanosomes. The history of such cultures is as follows:

> First day. No motile forms are seen.
>
> Second day. A number of motile cells appear which occupy the upper layer of sedimented corpuscles and which show macroscopically as whitish dots.
>
> Third day. A few motile forms are seen.
>
> Fourth day. Motile forms, typical trypanosomes, appear in numbers, multiply rapidly, and reach their maximum in from ten to fourteen days.

In cultures at room temperature the trypanosomes remain motile for forty-five days. At from 10° to 20° C. they live for three months. Subcultures are also readily made.

These trypanosomes Miyajima regarded as derivatives from the *Piroplasma* present, for the following reasons: In the first place, the blood of 200 cattle was examined for trypanosomes, without result, and trypanosomes have never been seen in Japanese cattle. He then selected 21 native animals and examined their blood. In 9 of these *Piroplasma* was found; in 12 it was not. Cultures were then made of the blood of these 21 animals, and trypanosomes appeared in 7 cases, all from animals the blood of which had previously shown *Piroplasma*. The remaining 14 cultures were negative.

Further to prove his results, Miyajima tried the following experiment: Three calves were selected the blood of which failed to show parasites. These were injected with a culture containing motile trypanosomes in abundance, and later were kept free from ticks. One animal gave negative results, but the other two became infected, as was proved first culturally and later microscopically. Miyajima writes (p. 90):

> In one instance, eight days after inoculation, the blood of the susceptible animal began to give a growth of the flagellated parasite in culture, whereas seventeen days later, by the aid of the microscope alone, the intracellular parasites were visible in the same animal.

It was found that the quantity of blood used to inoculate the tubes was a matter of indifference. One platinum loopful gave precisely the same results as 1 cubic centimeter.

Miyajima's conclusions, given on page 90, are here quoted:

> 1. A variety of hæmocytozoa known as *Piroplasma parvum* can readily be cultivated outside of the living body.
> 2. The parasites undergo the developmental change in blood bouillon and finally take the form of a typical *Trypanosoma*. This *Trypanosoma* can not be detected in the blood of living animals.
> 3. A simple mixture of blood and bouillon is the most suitable medium for the cultivation of protozoa such as *Piroplasma parvum* and *Trypanosoma lewisi*.

In an earlier communication Miyajima, in collaboration with Shibayama (Miyajima and Shibayama, 1906), brought out the following facts: From 13.6 to 76.1 per cent of Japanese cattle, according to locality, were found to be parasitized by a *Piroplasma*. It was not possible, by blood injections, to transfer this parasite from one animal to another. There was no evidence to show that this parasite is pathogenic to either native or foreign cattle. In the case of the former the only method whereby the presence of the parasite could be demonstrated was by microscopical examination. As to the latter, the authors examined a herd of 20 cattle imported some time previously from Wisconsin. Although all of these animals had remained in perfect health, 14 were found to be parasitized. On the other hand, two Japanese calves (age not given) were infested with ticks hatched from infectious eggs received from the Bureau of Animal Industry. The eggs were from ticks collected in Texas. The two calves, each infested with 200 ticks, responded by a mild attack of what was presumed to be Texas fever, but with regard to the blood examination the authors wrote: "Während die ganzen Beobachtungszeit zeigte der tägliche Parasitenbefund bei der Versuchstieren keine Abweichung von dem normalen Kalbes." From which the supposition is that *Piroplasma bigeminum* was not found, but that the Japanese *Piroplasma* remained present as in the control animals.

From the above it is evident that the Japanese *Piroplasma* found by these authors is peculiar in that it is not pathogenic. In this respect it differs from all of the other known species of this genus. Whether this is any warrant for believing that it is really not a *Piroplasma* at all is of course very questionable, but it may be pointed out that it does not follow that all of the pear-shaped parasites of the red blood cells of mammals are genetically connected. How this may be can not be determined until the life history of each species is known. I call attention to this fact here, since Miyajima's work is quoted as showing that *Piroplasma* has a trypanosome stage in its life history, a conclusion scarcely warranted.

In addition to the above papers, which are concerned with the attempt to link together trypanosomes and Hæmosporidia, there are a considerable number of observations which prove the presence of trypanosomes in cattle where there is no reason to believe them pathogenic.

Frank (1909), in Germany, found living specimens in the spleen pulp of an ox, the animal having died of a disease taken to be either anthrax or blackleg. The bacilli of neither of these diseases being found, Frank was inclined to blame the animal's death on the trypanosome, but no proof was furnished.

Frosch (1909) describes this parasite, which he calls *Trypanosoma* Frank, his material being stained smears from the spleen. It shows a sharply pointed posterior end, often terminated by a flagellum; a round, peripherally placed kinetonucleus, between which and the trophonucleus there is a wide interval. Frosch agrees with Frank in considering the trypanosome to be pathogenic.

Frank and Frosch (1909) restate the proposition of the pathogenicity of this parasite, saying that this is the first case of a bovine dying in Europe of trypanosomiasis.

Knuth (1909), studying the same material, made measurements of the flagellates present. He gives the length as from 20 to 40 μ, which includes the flagellum; the breadth as 2 μ. According to this author, it more closely resembles *T. theileri* than any other species.

Mayer (1909) also discusses this same trypanosome, and after going over its morphology says that it has the characters of a certain group of trypanosomes, typified by *Trypanosoma theileri*, which are found exclusively in cattle, hitherto only in tropical or subtropical countries.

Mayer then quotes a number of authors who have found trypanosomes occurring sporadically in cattle. Such mostly show the characters of this group. These discoveries were made in Asia and Africa. In some cases the trypanosomes were in healthy cattle; in others in those suffering from various diseases other than trypanosomiasis. Among these may be mentioned piroplasmosis and rinderpest. In no instance was there any reason to look upon the trypanosomes as pathogenic.

Hence Mayer regards the animal discovered by Frank to be harmless. Finally, he calls it *Trypanosoma franki*, a valid name.

METHODS.

Miyajima's work was repeated in this laboratory with entirely satisfactory results. Bovine blood, cultured in common beef bouillon, develops trypanosomes in from two to four days, dependent upon temperature. They also appeared in cultures of cow's blood in mutton bouillon, either acid or alkaline, and, furthermore, they developed in the case of every cow tested.

The methods were as follows: Blood was drawn from the jugular vein of the cow by means of a syringe, and transferred to flasks of 100 c. c. capacity. About 30 to 50 c. c. of blood was taken in each case. In each flask were placed 6 to 8 facetted beads of rough glass, such as those found in shops, strung together, for sale as very cheap necklaces. A few minutes shaking of the flask serves to collect the fibrin into a solid clot, embedded in which will be the beads. In these operations suitable precautions were taken to prevent contamination.

To take the blood from the flasks, pieces of glass tubing were used, one-fourth inch in diameter and 8 to 10 inches long. Over one end of each of these a piece of rubber tubing was pulled, to serve as a mouthpiece. The quantity of blood desired may then be drawn up into the glass tube by suction. The tubes with the rubber tubing attached were sterilized by boiling for five to ten minutes and then used at once. Owing to the poor conductivity of glass, they do not cool readily, and it was considered possible that they might heat the blood sufficiently to kill any animal life present. Accordingly, the precaution was usually taken of allowing the first filling of the glass tube to drop back into the flask and of using only the second.

As soon as the glass tube was filled with the blood to be used, the rubber tube was closed by pinching and the contents transferred at once to a culture tube. This operation was always performed by two persons, one to handle the blood, the other the culture tubes. In this way the latter were not exposed to the possibility of contamination for more than a second or two.

This procedure gave entirely satisfactory results. Bacterial contamination, when it occurs, appears to be the result either of getting the blood contaminated during the process of drawing it, or in the later handling of the tubes. Moreover, it is not quite so destructive to the growth of the trypanosomes as some authors maintain. While the contaminating of cultures is a reflection on the operator's technique, the trypanosomes seem able to endure a certain amount of plant life, and may even be found alive in very foul cultures. It was also at times noted that bacteria would start in a tube and later die out.

OBSERVATIONS.

As a rule the trypanosomes appeared on the third day. In one case, however, the "room temperature" being high, from 80° to 90° F., they were found in forty hours. After a few days the microscope is not necessary to demonstrate their presence, since they collect in little colonies on the surface of the column of blood cells.

These colonies show as small, white plates, and may be 3 or 4 millimeters in diameter. They are readily distinguishable from bacterial contamination, in that they are flat and sharply circumscribed, whereas the masses of bacteria are always more or less diffuse and tend to cloud the bouillon.

The ratio of the quantity of blood to bouillon was varied within wide limits, but, as Miyajima found, this makes no difference as far as the appearance of trypanosomes is concerned.

So far as the study has yet gone, the flagellates have appeared in the blood of all the cows tested, seven in number. Of these, the first used was a cow born at the Experiment Station of the Bureau of Animal Industry at Bethesda, Md., on November 15, 1900. This animal had twice been injected with blood from cattle known to be parasitized with *Piroplasma bigeminum.* It was hence assumed that her blood contained this parasite, and it was for this reason that she was chosen for the experiment.

Accordingly, the appearance of the trypanosomes was taken to be an exact confirmation of Miyajima's results, to the effect that *Piroplasma* has a flagellate stage in its life history. But whereas the Japanese biologist had to depend upon the tedious and uncertain method of examination to determine whether or not he was dealing with animals parasitized by *Piroplasma,* this point can in the United States be settled without difficulty and with almost the certainty of a mathematical demonstration. The cattle in the northern parts of the United States, outside of the tick-infested region, are free from *Piroplasma.* This can be proven in individual cases by placing infectious ticks on the animal—i. e., ticks which have hatched from eggs laid by ticks taken from southern cattle. A northern cow thus treated will sicken of Texas fever and usually die, whereas no effects will be noticed in the case of a cow in the blood of which *Piroplasma* was already present.

Therefore, four animals were obtained from north of the Federal quarantine line, and cultures made of their blood. The trypanosomes appeared with due promptness. Since the chances were all against these animals being parasitized by *Piroplasma* (and, further, since one of them, as noted below, after being infested with ticks contracted Texas fever with fatal results), it was seen that there was a serious discrepancy between Miyajima's results and mine. My Japanese colleague got trypanosomes from blood in which he demonstrated the presence of a *Piroplasma,* and failed to get them in blood in which *Piroplasma* could not be found, whereas my experience is that the trypanosomes appear without reference to the presence or absence of Hæmosporidia.

As an additional test, two cows from the herd at the Experiment Station of the Bureau of Animal Industry were tried, and the flagellates developed in cultures from their blood. The animals of this herd are assumed to be, and in all probability are, free from *Piroplasma bigeminum.* Finally, one of the four cows purchased for the experiment was infested with infectious ticks, and in due course died of Texas fever—practically absolute proof that at least one animal from the blood of which trypanosomes were cultured did not originally harbor the hæmosporidian. It was not considered necessary to sacrifice the other animals used in the study.

The first point which suggests itself is whether the trypanosomes which appear in culture tubes arise from actual trypanosomes originally present in the blood or from some element of a wholly different facies. While this point can not be regarded as finally settled, the probabilities are in favor of the second hypothesis. The blood of American cattle, in both health and disease, has been studied for many years, and with a single exception (Bowhill, 1909) trypanosomes have never been reported. If they were normally present in any numbers, it seems unlikely that they should so far have escaped notice. Moreover, while many of the cultures were examined daily from the first, flagellates were never found on the first day, and but once on the second.

But the most direct evidence that they evolve in the tubes from some unrecognized form is the fact that at first we do not find typical trypanosomes, or even flagellates, but round or oval bodies. Miyajima figures bodies which he regards as the forerunners of the flagellates, but it is not certain that these are not white blood cells. Neither his figures, unfortunately made by photography, nor his description permit of a final judgment.

In my own preparations I have now and then come across an isolated spherical body, of much the size of a large leucocyte, which does not exactly resemble any of the various types of white cells. It may be that such belong to the cycle of the trypanosomes, but to settle this requires further investigation. At present I am only able to say that I have not been able to get hold of anything which can safely be put down as a forerunner of the trypanosomes until the body shown in figure 3, *a*, appears.

This condition may be seen in young cultures. Such masses consist of closely compacted cells which are probably round or oval, but which are sometimes polyhedral on account of mutual pressure. They stain blue in Wright's stain, and each shows a distinct kinetonucleus, but trophonuclei are not to be made out. Clusters of this sort vary greatly in size. They may consist of as few as three or four organisms or as many as several hundreds.

This leads up into the condition shown in figure 3, *b*. Here is a cluster of little flagellates, of which the cytoplasm is still sharply blue and the kinetonuclei very distinct. Flagella may or may not be present. Apparently the organism evolves from a rounded cell into a lenticular flagellate, but in some cases the lenticular form may be assumed before the flagellum has differentiated. Small, isolated lenticular organisms are often seen which do not show flagella.

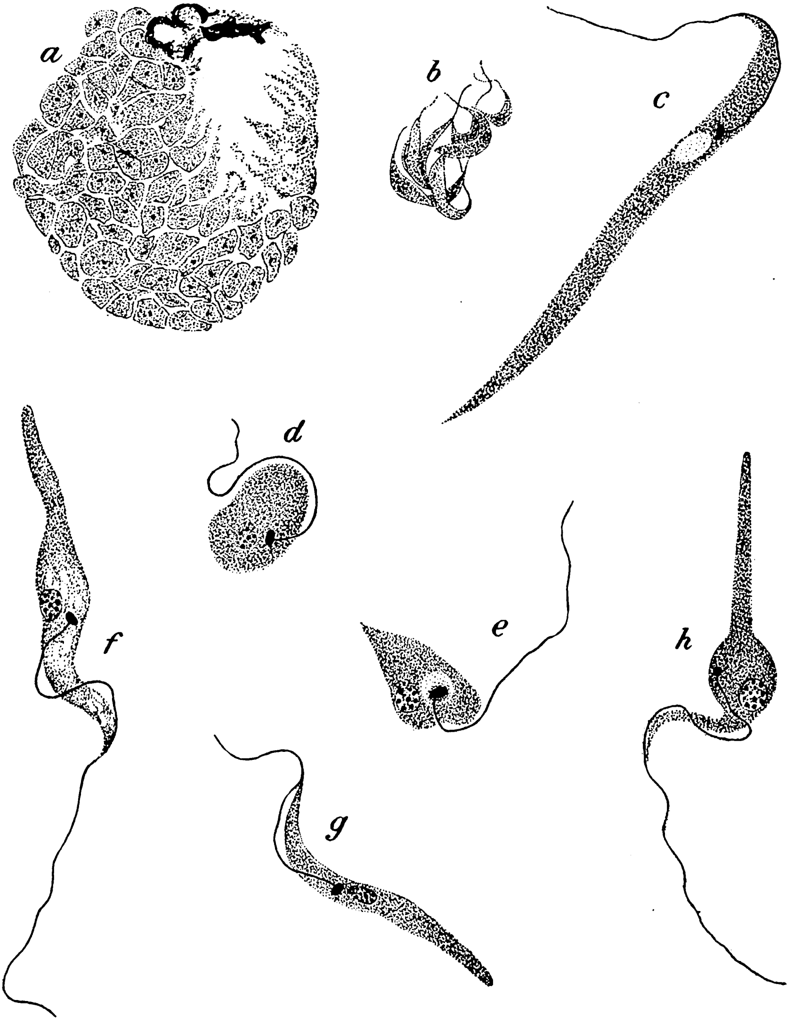

FIG. 3.—*Trypanosoma americanum* n. sp. *a*, cluster of young forms, 80-hour culture, × 800; *b*, cluster of small flagellated forms, 88-hour culture, × 800; *c*, crithidia stage, 88-hour culture, × 2,467; *d*, monadine form, 10-day culture, × 2,467; *e*, monadine form—the kinetonucleus lies in a vacuole stained pink by Wright's stain, 10-day culture, × 2,467; *f*, typical band-shaped trypanosome, with fully developed undulating membrane, 10-day culture, × 2,467; *g*, small band-shaped trypanosome, with fully developed undulating membrane, 10-day culture, × 2,467; *h*, club-shaped form, with fully developed undulating membrane, 10-day culture, × 2,467. (Figures made from camera outlines.)

As a rule, however, these little bodies are typical flagellates, resembling *Crithidia* in morphology, and it is convenient to speak of this developmental phase as the crithidia stage. From this point on, in at least a considerable proportion of the cases, evolution consists apparently in a mere increase in size, till we reach the condition shown in figure 3, *c*. Here we have a long, band-shaped cell, with a sharply pointed posterior end. The specimen drawn was 28 μ long without the flagellum. The cytoplasm of these stages stains a clear blue, the kinetonucleus is very distinct, while the trophonucleus is represented by a pale, reddish vacuole, and is clearly very poor in nucleic acid. The flagellum is distinct, but an undulating membrane can not be distinguished in stained preparations.

In addition to the elongated crithidia forms, a monadine form also appears in young cultures, such as is depicted in figure 3, *d* and *e*. These monads become more abundant as the cultures grow older, but so far as my observations go at present they never form more than a small percentage of the trypanosomes present.

The typical trypanosomes are shown in figure 3, *f*, *g*, and *h*. In these the trophonucleus shows as a fair-sized vesicle containing coarse chromatin bodies. The kinetonucleus is usually elongated, forming a long ellipsoid, and stains an almost black garnet color. The two nuclei are always close together. The undulating membrane is well developed. As may be seen from the figures, the parasites appear under two forms, a band shaped and a club shaped. What significance this dimorphism may possess has not been worked out. It may be stated, however, that these two forms are readily to be distinguished from one another, although they to a certain extent intergrade. The band-shaped forms, or more typical trypanosomes, are more numerous than the others. At first glance the club-shaped phase might be looked upon as a degenerating condition, i. e., as a so-called involution form.[a] But this can hardly be the case. The club-shaped animals appear in the cultures as soon as do the others, and neither their appearance in life nor their staining reactions in smears carries the least suggestion of degeneracy.

The specimens drawn in figure 3, *f*, *g*, and *h*, were of intermediate size. Larger individuals were seen, and the length over all may reach as much as 60 μ. In cases where degeneracy had apparently set in the posterior end was often greatly prolonged and now and then provided with a flagellum.

Division, in the cases noted, takes place by equal, longitudinal fission.

It will be seen from the above that the trypanosomes which appear in the culture tubes do not arise, so far as all appearances go, from preexisting trypanosomes, but from larger or smaller clusters of round or oval cells, which in their turn are doubtless derived from some unrecognized form. Since the study is as yet by no means complete, it is necessary to speak with all reserve; but as long as the earlier cultures show only the clusters of rounded cells or flagellates of the crithidia type we appear to have a development from some condition which bears no resemblance to a flagellate, and which has not yet been recognized. It is only in the somewhat older cultures that typical trypanosomes appear, but these older cultures also show the adolescent stages, suggesting that all of the hypothetical germs present in a tube do not develop at the same rate.

---

[a] The use of the term "involution" as a synonym for "degenerate" is extremely unfortunate, but it probably has obtained too good a foothold in the literature to permit of the hope of its being dropped.

In all the cultures the trypanosomes tend to occur in great clusters. Thus such a condition as is shown in figure 3, *a*, appears to give rise to a similarly constituted mass of trypanosomes in either the crithidia or trypanosome stage. The trypanosomes occurring free in the liquid are apparently individuals which have escaped from such colonies. These clusters, however, are not to be confounded with agglutination rosettes, which present a wholly different appearance. In the clusters nothing in the way of a definitive orientation can be made out, whereas in all the agglutinations seen the arrangement was radial.

Observations on the living trypanosomes were made with either ordinary light or the dark-field illumination. They perish so quickly with the latter, however, that its use is scarcely to be recommended. The trypanosomes are energetic enough, but seldom display a progressive movement. When they do, however, it is very rapid and may be either forward or backward. For the most part, however, the movements are confined to vigorous lashings of the flagellum, changes of the form of the body, and a sort of jumping hither and yon, which does not bring about any extensive change of shape. The club-shaped forms were often seen to display a very quick, sudden, jerky movement, accompanied by a shift in the direction of the longitudinal axis. It was like the movement frequently displayed by *Stylonychia* and related ciliates, only much quicker and more abrupt.

When the fresh mounts were of sufficient thickness as not to compress the large clusters, these, in mass, were seen to change outlines like slow amebas, and to shift their positions. They are covered with a mantle of very lively flagella and are so compact that they suggest a metazoan as much as they do a cluster of protozoans.

It may be concluded that the trypanosome here described and figured is a common parasite of healthy American cattle. Its morphological peculiarity is that the trophonucleus and kinetonucleus lie very close together. This peculiarity is shown by *T. transvaliense*, taken to be a variety of *T. theileri*, and, as well as can be made out from his figures, by the trypanosome found by Miyajima. If this last fact be so, then Miyajima is in error in his conclusion that his flagellate is a phase of *Piroplasma*. At all events, the fact that trypanosomes appear in cultures of blood from healthy cattle is of considerable significance, and is decidedly against the belief that they are stages in the life history of a hæmosporidian.

Upon the completion of further investigations concerning this trypanosome, for which the name *Trypanosoma americanum* is suggested, a more extended account of its morphology and ontogeny will be given.

BIBLIOGRAPHY.

Bowhill, Thomas.

1905.—Equine piroplasmosis, or "biliary fever"<J. Hyg., Cambridge, v. 5 (1), Jan., pp. 7–17, pls. 1–3, figs. 1–15.

1909.—Note on the occurrence of a large flagellate, associated with piroplasmata infection in a cow in British Columbia. [Extract from Vet. News, Aug. 15, 1908, p. 474] <J. Trop. Vet. Sc., Calcutta, v. 4 (1), pp. 104–105, 1 fig.

Bowhill, Thomas; & Le Doux, C. A.

1904.—A contribution to the study of piroplasmosis canis—malignant jaundice of the dog (Hutcheon) <J. Hyg., Cambridge, v. 4 (2), Apr., pp. 217–218, pl. 11, figs. 1–6.

Frank, G.

1909.—Ueber den Befund von Trypanosomen bei einem in Stein-Wingert (Westerwald, Regierungsbezirk Wiesbaden) verendeten Rinde <Ztschr. f. Infektionskr. . . . d. Haustiere, Berl., v. 5 (3–4), 10. Feb., pp. 313–315.

Frank, G. & Frosch, P.

1909.—Ueber die Bedeutung des Befundes rinderpathogener Trypanosomen in Deutschland <Ztschr. f. Infektionskr. . . . d. Haustiere, Berl., v. 5 (3–4), 10. Feb., pp. 330–334.

Frosch, P.

1909.—Aetiologische Ermittelungen über das *Trypanosoma* Frank <Ztschr. f. Infektionskr. . . . d. Haustiere, Berl., v. 5 (3–4), 10. Feb., pp. 316–329, pls. 10–13, figs. 1–8.

Knuth, P.

1909.—Ueber die Morphologie des Trypanosoma Frank <Ztschr. f. Infektionskr. . . . d. Haustiere, Berl., v. 6 (1), 18. Mai, pp. 39–45, 1 fig.

Mayer, Martin.

1909.—Ueber *Trypanosoma theileri* und diesem verwandte Rindertrypanosomen <Ztschr. f. Infektionskr. . . . d. Haustiere, Berl., v. 6 (1), 18. Mai, pp. 46–51, pl. 1, figs. 1–2.

Minchin, E. A.

1909.—Protozoa <Syst. Med. (Allbutt & Rolleston), Lond., v. 2, pt. 2, pp. 9–122, figs. 1–82.

Miyajima, M.

1907.—On the cultivation of a bovine piroplasma: A preliminary communication <Phillipine J. Sc., Manila, B. Med. Sc., v. 2 (2), May, pp. 83–92, pls. 1–3, figs. 1–12.

Miyajima, M.; & Shibayama, G.

1906.—Ueber das in Japan beobachtete Rinderpiroplasma <Ztschr. f. Hyg. u. Infektionskr., Leipz., v. 54 (2), 11. Sept., pp. 189–200, pl. 8, figs. 1–20.

Schaudinn, Fritz.

1904.—Generations- und Wirtswechsel bei *Trypanosoma* und *Spirochæte*. (Vorläufige Mitteilung) <Arb. a. d. k. Gsndhtsamte., Berl., v. 20 (3), pp. 387–439, figs. 1–20.

O